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14. ABSTRACT The overall objective of our work on human ovarian carcinoma cells is to apply our findings on the role of hyaluronan-CD44-CD147 interactions in drug resistance to improvement of therapy for malignant ovarian carcinoma. In previous work we showed that small hyaluronan oligosaccharides sensitize drug-resistant human ovarian carcinoma cells to various chemotherapeutic agents in culture and in vivo. In this grant period, we have: a) shown that drug-resistant Hey-A8-MDR human ovarian carcinoma cells contain CD133-positive/ CD147-positive/ CD44-positive cancer stem-like cells, as are also found in human patient ascites-derived ovarian carcinoma cells, thus documenting their suitability for our studies; b) completed preparation of hyaluronan oligosaccharide conjugated to the chemotherapeutic agent, docetaxel; we anticipate that this conjugate will show increased efficacy in vivo; c) identified a highly active siRNA against the regulator of hyaluronan synthesis, CD147, that sensitizes Hey-A8-MDR ovarian carcinoma cells to docetaxel and is currently being used to load nanoparticles for higher efficacy in vivo.

15. SUBJECT TERMS
Ovarian carcinoma; hyaluronan; CD147; CD44; drug resistance

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INTRODUCTION

Our previous studies have shown that hyaluronan, the hyaluronan receptors CD44 or LYVE-1, and the Ig superfamily member CD147 act cooperatively to promote malignant and drug-resistant properties. This most likely occurs through assembly and/or stabilization of plasma membrane signaling complexes containing CD44 or LYVE-1 in association with CD147, receptor tyrosine kinases and transporters implicated in malignancy and resistance to therapies (Ghatak et al., 2005; Grass et al., 2013; Qin et al., 2011; Slomiany et al., 2009a; Slomiany et al., 2009b; Slomiany et al., 2009c). CD147 (emmprin; basigin) is a cell surface member of the Ig superfamily that induces expression of hyaluronan and matrix metalloproteinases, and promotes cell invasiveness, anchorage independent growth, drug resistance, and tumor growth and metastasis *in vivo* (Caudroy et al., 2002; Dai et al., 2013; Grass et al., 2012; Marieb et al., 2004; Zucker et al., 2001). We have shown recently that sub-populations of ovarian carcinoma cells, and other cancer cell types, with high expression of cell surface CD147 have similar properties to cancer stem cells, including enhanced levels of anchorage-independent growth, drug resistance and invasiveness (Dai et al., 2013).

Many of the activities of CD147 in cancer cells are dependent on hyaluronan-CD44 or LYVE-1 signaling (Ghatak et al., 2005; Grass et al., 2013; Marieb et al., 2004; Misra et al., 2003; Qin et al., 2011) and CD44 is one of the most common markers for carcinoma cancer stem cells (Zoller, 2011). The overall objectives of our work are to determine the mechanisms whereby hyaluronan-CD44-CD147 interactions influence malignant cell behavior and therapy resistance, and to apply our findings to the improvement of therapy, in particular in recurrent ovarian carcinoma. For example, we have found that multivalent interactions of hyaluronan polymer with CD44 are necessary for stabilizing CD44-CD147 signaling complexes, and that small, monovalent, hyaluronan oligosaccharides antagonize hyaluronan-receptor signaling by disrupting constitutive hyaluronan polymer-receptor interaction, thus leading to inhibition of oncogenic signaling pathways, chemoresistance and malignant characteristics (Ghatak et al., 2002; Ghatak et al., 2005; Gilg et al., 2008; Grass et al., 2013; Misra et al., 2006; Qin et al., 2011; Slomiany et al., 2009a; Slomiany et al., 2009b; Slomiany et al., 2009c). In particular we have found that treatment with small hyaluronan oligosaccharides is effective in sensitizing various types of drug-resistant cancer cells to chemotherapeutic agents (Gilg et al., 2008; Misra et al., 2005; Misra et al., 2003; Qin et al., 2011; Slomiany et al., 2009a). Most notably, these oligosaccharides inhibit tumor growth by drug-resistant cancer stem cell sub-populations obtained from human patient-derived ovarian carcinoma cells (Slomiany et al., 2009b).

Our aims for this grant are to establish efficacy for small hyaluronan oligosaccharides as chemo-sensitizing agents in xenograft models of human ovarian carcinoma cells, to determine whether siRNA directed against CD147 or CD44 affect ovarian carcinoma chemoresistance and metastasis, and to explore mechanisms for increasing efficiency of delivery of these agents together with chemotherapeutic agents.

KEYWORDS
Ovarian carcinoma; hyaluronan; CD147; CD44; drug resistance

OVERALL PROJECT SUMMARY

In this grant period, our major aims have been:

a) To show that drug-resistant human ovarian carcinoma cell lines and human patient ascites-derived ovarian carcinoma cells contain CD133-positive/ CD147-positive/ CD44-positive cancer cells; we have shown previously that cells with these markers have the properties of cancer stem-like cells (Dai et al., 2013)(Slomiany et al., 2009b);

b) To prepare hyaluronan oligosaccharides conjugated to the chemotherapeutic agent, docetaxel - we anticipate that these conjugates will show increased efficacy in vivo;

c) To identify a highly active siRNA against the regulator of hyaluronan synthesis, CD147, and to use this siRNA to load nanoparticles for higher efficacy in vivo.

a) Fluorescence-activated cell sorting (FACS) analyses of human ovarian carcinoma cells

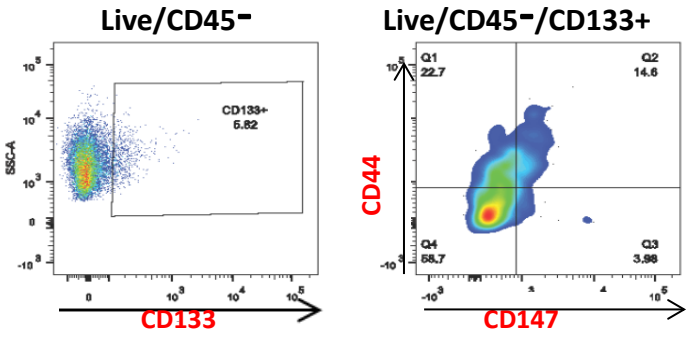
We analyzed SKOV3 human ovarian carcinoma cells, drug-resistant Hey-A8-MDR human ovarian carcinoma cells and patient ascites-derived primary human ovarian carcinoma cells by FACS. In the case of the primary ascites-derived cells, we first separated the cells from ascites fluid by low speed centrifugation and then removed red blood cells. We then incubated the remaining cells in tissue culture dishes for a short period – the rapidly attaching cells, mainly fibroblasts, were discarded. The suspended cells were re-plated for 24 hours during which the cancer cells attached but most leucocytes did not. The attached cells were washed and used for FACS analysis as described previously (Slomiany et al., 2009b).

SKOV3 cells, Hey-A8-MDR cells and ascites-derived cells were subject to FACS analysis in the same manner, as follows. We first separated live cells from dead cells, and then separated CD45-negative cells from CD45-positive, putative leucocytes. The CD45-negative cells were then split into CD133-negative and CD133-positive sub-populations. From past data from this and other laboratories (Slomiany et al., 2009b), we expect the CD133-positive cells to be enriched in cancer stem-like cells. For all three cell types, approximately 5% of the CD45-negative cells were CD133-positive (Fig. 1). Since CD44 and CD147 are also associated with cancer stem cells, we then analyzed the distribution of CD44 and CD147 in the CD133-positive sub-population. For both the SKOV3 and Hey-A8-MDR cells, greater than 90% of the CD133-positive, cancer stem-like cells were also positive for CD44 and CD147 (Fig. 1B,C). However, in the case of the ascites-derived cells, ~15% were CD44-positive and CD147-positive, ~23% were CD44-positive/ CD147-negative, and ~4% were CD147-positive/ CD44-negative (Fig. 1A).

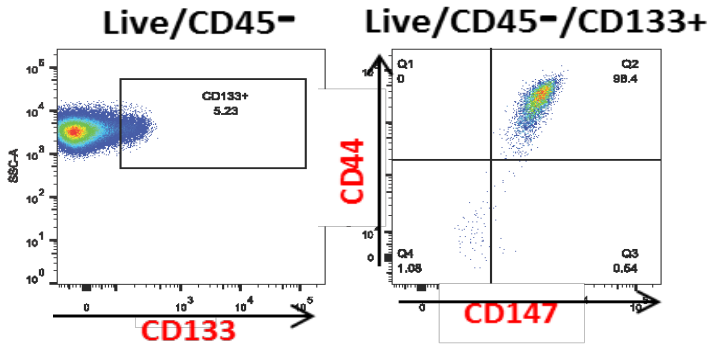
These results suggest strongly that SKOV3 and Hey-A8-MDR cells are enriched in a sub-population of cells with highly cancer stem-like properties. Primary ascites cells also contain similar cells but at lower proportions.

FIGURE 1

A. Ascites cells



B. SKOV3 cells



C. Hey-A8-MDR cells

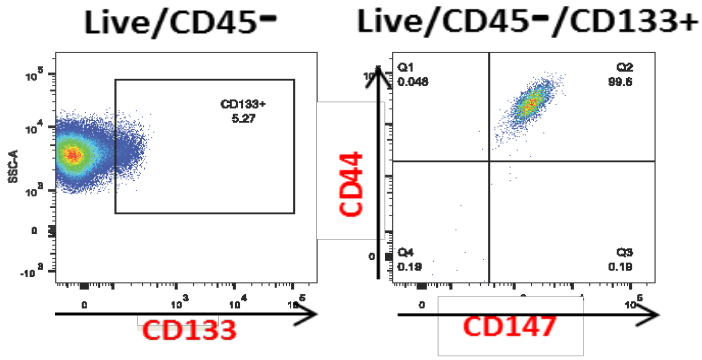


Figure 1. FACS analyses of ovarian carcinoma cells. A. Patient-derived ascites cells; B. SKOV3 cells; C. Hey-A8-MDR cells.
In each case, dead cells and CD45-positive cells were eliminated from the analyses. CD133-positive/negative cells were analyzed within the live cell/CD45-negative population, and then the CD133-positive cells were analyzed for CD147 and CD44. In each case, ~5% of the live cell/CD45-negative population was CD133-positive. Greater than 90% of the CD133-positive SKOV3 or Hey-A8-MDR cell population was CD147-positive and CD44-positive. However, only ~15% of the CD133-positive ascites cell population was CD147-positive and CD44-positive.

b) Hyaluronan oligosaccharide-conjugated docetaxel

In collaboration with Drs. Dahai Jiang, Selanere Mangala and Anil Sood (MD Anderson Cancer Center), we have prepared docetaxel conjugated with small hyaluronan oligosaccharides. After several preliminary experiments, we have now prepared a large scale batch of these conjugates for use in xenograft experiments. We anticipate that these conjugates will show greater efficacy in vivo than simple mixtures of docetaxel and hyaluronan oligosaccharide as used previously. The hyaluronan oligosaccharide will bind CD44 in such a manner as to inhibit the activity of endogenous hyaluronan-CD44-CD147-receptor tyrosine kinases/ transporter complexes (Ghatak et al., 2005; Grass et al., 2013; Qin et al., 2011; Slomiany et al., 2009a; Slomiany et al., 2009b; Slomiany et al., 2009c), and induce internalization of the docetaxel conjugate along with CD44 (Qin et al., 2011; Slomiany et al., 2009a; Slomiany et al., 2009b; Slomiany et al., 2009c).

c) CD147 siRNA-loaded nanoparticles

Also in collaboration with Drs. Jiang, Mangala and Sood, we are preparing liposomes containing siRNA against CD147. We anticipate that these liposome particles will increase the efficacy of action of siRNA against CD147 in similar manner to the approach previously used by the Sood laboratory (Landen et al., 2010; Mangala et al., 2009; Spannuth (Graybill) et al., 2011). Despite earlier difficulties with obtaining efficient knockdown, we have now identified siRNAs against CD147 that are very efficient (Fig. 2). These siRNAs, especially siRNA2 (Fig. 2), are now being used to manufacture the liposomal delivery nanoparticles for use in xenograft experiments.

FIGURE 2

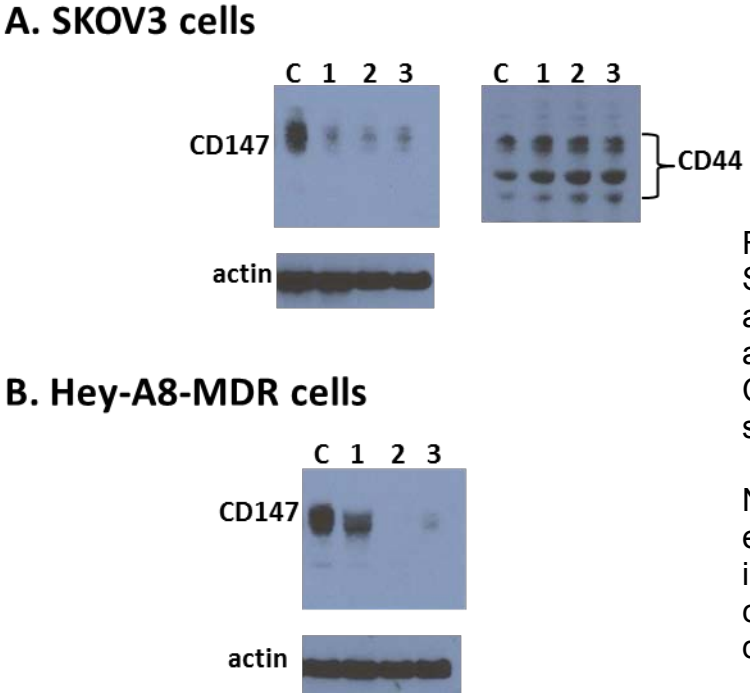


Figure 2. Western blot analyses of SKOV3 and Hey-A8-MDR cells after treatment with siRNAs against CD147.
C: control siRNA; 1,2,3: siRNA1,2,3 against CD147.

Note that siRNA2 and 3 are very effective in knocking down CD147 in both SKOV3 and Hey-A8-MDR cells. These siRNAs did not knock down CD44.

KEY RESEARCH ACCOMPLISHMENTS

1. Demonstration of CD133/ CD44/ CD147-positive cancer stem-like cells in SKOV3 cells, Hey-A8-MDR cells and primary human ovarian carcinoma cells derived from patient ascites.
2. Preparation of hyaluronan oligosaccharide-conjugated docetaxel to increase efficacy *in vivo*.
3. Demonstration of almost complete knockdown of CD147 with siRNAs, which are being used for loading of liposome particles that are designed for increased efficacy *in vivo*.

CONCLUSIONS

We have shown that human ovarian carcinoma cell lines contain a sub-population of cancer stem-like cells, which is characterized by high levels of CD133, CD147 and CD44 and which is also found in primary human ovarian carcinoma cells. We have prepared small hyaluronan oligosaccharides conjugated to docetaxel and liposomes loaded with an effective siRNA against CD147 that are designed to inhibit the previously demonstrated hyaluronan-CD44-CD147 axis more efficiently than in simple reagent mixtures. Recurrence of ovarian cancer in patients who have been given standard-of-care chemotherapy is a major cause of patient morbidity. Thus further investigation of more efficient methods of treatment of drug resistance is an important objective.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

None

INVENTIONS, PATENTS AND LICENSES

None

REPORTABLE OUTCOMES

None

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